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CHEMICAL ABSTRACTS, vol. 94, no. 3, 19th January 1981, page 177, abstract no. 12553m, Columbus, Ohio, US; G.-L. YIN et al.: "Studies on the production of vitamin C precursor-2-keto-L-gulonic acid from L-sorbose by fermentaion. I. Isolation, screening and identification of 2-keto-L-gulonic acid producing bacteria"

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Descripti n

The present invention relates to a fermentation process, i. . to a process for producing 2-keto-L-gulonic acid by fermentation. Furthermore, the invention relates to certain microorganisms which can be used in such process.

2-Keto-L-gulonic acid is an important intermediate for the production of ascorbic acid into which it can be converted according to the well-known Reichstein method.

The fermentative production of 2-keto-L-gulonic acid from D-sorbitol or L-sorbose is known.

Thus, Japanese Patent Publication No. 40154/1976 discloses the production of 2-keto-L-gulonic acid from D-sorbitol by means of microorganisms of the genus Acetobacter, Bacterium or Pseudomonas, which microorganisms are capable of oxidizing D-sorbitol under aerobic conditions, thus producing 2-keto-L-gulonic acid. The yield of this known process is, however, rather low, namely less than 6 g/l.

According to another known process, which is disclosed in "Acta Microbiologica Sinica" 21(2), 185-191, (1981). 2-keto-L-gulonic acid can be produced from L-sorbose by means of a mixed culture of microorganisms, which comprises Pseudomonas striata and Gluconobacter oxydans, the yield being 30 g/l when starting from a concentration of 70 g/l of sorbose, and 37 g/l when starting from a concentration of 100 g/l of sorbose.

According to the present invention, it is possible to produce 2-keto-L-gulonic acid from L-sorbose at a much higher yield, namely a yield of more than 40 g/l and even more than 50 g/l, when starting from a sorbose concentration of 70 g/l, and at higher yields when starting from higher concentrations.

The instant process for producing 2-keto-L-gulonic acid by conversion of L-sorbose by means of mixed cultures of microorganisms is characterized in that a mixed culture of microorganisms comprising Gluconobacter oxydans and Bacillus megaterium is used.

We have denominated and classified the first one of these two microorganisms as Gluconobacter oxydans by reference to Bergey's Manual of Determinative Bacteriology, 8th edition, 1974, and, in particular, in view of the fact that it exhibits the following characteristics:

a) 2-Keto-L-gulonic acid is produced from sorbose,
b) ethanol is oxidized to acetic acid,
c) D-glucose is oxidized to D-gluconic acid and 2-keto-D-gluconic acid,
d) ketogenesis of polyalcohols,
e) pellicle and ring growth in mannitol broth (24 hrs cultivation) at pH 4 and 5, and pellicle growth in glucose broth at pH 4,5.

In addition to the above, it exhibits the following properties:

f) glycerol is not substantially oxidized to dihydrooxyacetone,
g) 2-keto-D-glucaric acid is produced from sorbitol and glucaric acid, but not from glucose, fructose, gluconic acid, mannitol or 2-keto-D-gluconic acid,
h) polymorphic, apparently no flagella,
i) brown pigment is produced from fructose,
j) good growth when co-cultured in the presence of Bacillus megaterium or a cell extract thereof,
k) streptomycin sensitive.

The second one of these two microorganisms was classified in view of the fact that it exhibits morphological, physiological, cultural and other characteristics typical for Bacillus megaterium.

Any strains belonging to the species Gluconobacter oxydans on the one hand and Bacillus megaterium on the other hand, isolated from natural sources or obtained from publicly available collections may be useful for being employed for the instant purpose, provided they are able, in the form of a mixed culture, to convert L-sorbose to 2-keto-L-gulonic acid with a satisfying yield, i.e. a yield of more than 40 g/l, particularly of at least 50 g/l and more particularly of at least 80 g/l.

A preferred mixed culture for use in the instant process for producing 2-keto-L-gulonic acid is culture No. 2980 or a subculture, mutant or variant thereof. Culture No. 2980 was deposited under DSM No. 4027 at the Deutsche Sammlung von Mikroorganismen in Göttingen on March 17, 1987. Note: The present address of Deutsche Sammlung von Mikroorganismen is: Mascheroder Weg 1b, D-3300 Braunschweig, Federal Republic of Germany.

This mixed culture is composed of a Gluconobacter oxydans strain exhibiting the characteristics set forth under a) to k) above, and a Bacillus megaterium strain.

A specific and preferred Gluconobacter oxydans strain (internal designation of Academia Sinica = AS. 1.945) has been deposited at the Center for General Microbiological Culture Collection, Institute of Microbiology, Zhong Guan Cun, Beijing, China, under the designation CGMCC No. 0119 on February 7, 1987. A subculture of this strain has been deposited at the Deutsche Sammlung von Mikroorganismen in

Göttingen under DSM No. 4025 on March 17, 1987.

A specific and preferred Bacillus megaterium strain (internal designation of Academia Sinica AS. 1.1484) has been deposited at the Center for Central Microbiological Culture Collection, Institute of Microbiology, Zhong Guan Cun, Beijing, China, under the designation CGMCC No. 0120 on February 7, 1987. A subculture of this strain has been deposited at the Deutsche Sammlung von Mikroorganismen in Göttingen under DSM No. 4026 on March 17, 1987.

The cells of both the Gluconobacter oxydans strain and the Bacillus megaterium strain are rod-shaped with rounded ends. The diameter of a cell of the Gluconobacter oxydans strain is, on the average about 0.3-0.6 µm, its length about 0.9-1.6 µm, mainly 1-1.5 µm. The diameter of a cell of the Bacillus megaterium strain is, on the average, about 1 µm-1.5 µm and its length about 2.0-5.0, mainly 4 µm. The two strain types can be easily differentiated by the above dimensions.

The quantative ratio of Bacillus colonies to Gluconobacter colonies at the beginning of the fermentation process is not critical. This ratio may e.g. be in the range between 1:10 and 1:300 (Bacillus:Gluconobacter). This ratio adjusts itself automatically, in the course of the fermentation process, to an optimal value.

The production of 2-keto-L-gulonic acid in accordance with the present invention is effected by cultivating the mixed microorganism culture referred to above in a medium containing L-sorbose as well as appropriate nutrients. Alternatively, the instant process may be carried out by culturing the mixed microorganisms referred to above and, thereafter, bringing the whole cells or a cell-free extract collected from the culture into contact with L-sorbose.

Where the mixed microorganisms are cultured in a medium containing L-sorbose as well as appropriate nutrients, the microorganisms are conveniently cultured in an aqueous medium under aerobic conditions.

The instant fermentation process may be carried out at a pH between about 5 and 8, preferably between about 6 and 8.

A preferred temperature range for carrying out the instant fermentation process is between about 25° and 35°C. More preferably, the instant fermentation process is carried out at 30° ± 1°C.

While the fermentation period may vary, depending on pH, temperature and nutrient medium used, usually 1/2 to 10 days will bring about favourable results.

The concentration of the L-sorbose substrate used as starting material in the instant process may vary between about 20 and 200 g/l, preferably between about 50 and about 100 g/l.

The culture medium used in the instant fermentation process usually contains such nutrients for the microorganisms as assimilable carbon sources, digestible nitrogen sources and inorganic substances, vitamins, trace elements and other growth promoting factors. In addition to the L-sorbose used as starting material in the instant process, other substances which are carbon sources may also be added, such as glycerol, glucose, mannitol, fructose, D-arabitol and the like.

Various organic or inorganic substances may be used as nitrogen sources in the instant process, such as yeast-extract, meat extract, peptone, casein, corn-steep liquor, urea, amino acids, nitrates, ammonium salts and the like. As inorganic substances, magnesium sulfate, potassium phosphate, ferrous and ferric chlorides, calcium carbonate and the Like may be used.

In the case, where pregrown whole cells collected from the culture, are used, the cultivation of the microorganisms is carriad out under the same or similar conditions as described above. These whole cells are used in an aqueous medium under aerobic conditions, no additional nutrients (in addition to the L-sorbose used as starting material) are necessary.

In case where cell-free extracts from the culture are used, these extracts are added to the substrate in an aqueous medium and are used in the conversion of L-sorbose to 2-keto-L-gulonic acid under aerobic conditions in a manner similar to that set forth above, no additional nutrients being necessary also in this case.

According to the present process it is possible to produce 2-keto-L-gulonic acid in a yield of at least 40 g/l, preferably at least 50 g/l, most preferably at least 80 g/l.

The 2-keto-L-gulonic acid obtained according to the present process can be isolated from the reaction mixture, e.g. by the formation of a salt or by using differences in properties between the product and the surrounding impurities, such as solubility, absorbability and distribution coefficient between the solvents. Adsorption, e.g. on ion exchange resins constitutes a convenient means for isolating the product. The thus obtained product may further be purified in a conventional manner, e.g. by recrystallization or chromatography.

Alternatively, the reaction mixture can be used directly for conversion to L-ascorbic acid by esterification, followed by enolization and lactonization.

The present invention also relates to a mixed microorganism culture which can be used in the above process and which is characterized in that it comprises microorganisms of the species Gluconobacter

oxydans and Bacillus megaterium.

For being useful in the above process, it is necessary that the above species are used in the form of a mixed culture, rather than individually.

A preferred mixed microorganism culture according to the instant invention is the cultur No. 2980 or a sub-culture, mutant or variant thereof. Culture No. 2980 was deposited under DSM No. 4027 at the Deutsche Sammlung von Mikroorganismen in Göttingen on March 17, 1987.

The mixed microorganism culture according to the instant invention is characterized by the ability to produce 2-keto-L-gulonic acid from L-sorbose in a yield of at least 40 g/l, preferably at least 50 g/l, most preferably at least 80 g/l.

The present invention also relates to the individual components of the mixed microorganism culture, i.e. to Gluconobacter oxydans cultures on the one hand and Bacillus megaterium cultures on the other hand, which, in combination, are able to meet the requirements set forth above for the mixed cultures, i.e. to convert L-sorbose to 2-keto-L-gulonic acid with a yield of more than 40 g/l, particularly of at least 50 g/l and more particularly of at least 80 g/l.

Representatives of such cultures are:

1. Gluconobacter oxydans culture AS. 1.945 (internal designation by Academia Sinica), deposited at the Center for General Microbiological Culture Collection, Institute of Microbiology, Zhong Guan Cun, Beijing, China, under the designation CGMCC No. 0119 on February 7, 1987, a subculture of which was deposited at the Deutsche Sammlung von Mikroorganismen in Göttingen under DSM No. 4025 on March 17, 1987.
2. Bacillus megaterium culture AS. 1.1484 (internal designation by Academia Sinica), deposited at the Center for General Microbiological Culture Collection, Institute of Microbiology, Zhong Guan Cun, Beijing, China, under the designation CGMCC No. 0120 on February 7, 1987, a subculture of which was deposited at the Deutsche Sammlung von Mikroorganisman in Göttingen under DSM No. 4026 on March 17, 1987.

Further representatives of such cultures are sub-cultures, mutants and variants thereof.

Mutants can be derived from the parent strains by conventional methods, e.g. irradiation with u.v., X- and gamma rays or by treatment with appropriate mutagens.

The present invention is illustrated by the following Examples:

Example 1

Fermentation in a 15 l laboratory fermentor

The mixed culture of Gluconobacter oxidans and Bacillus megaterium was streaked out on an agar Petri dish containing the ingredients of the seed culture medium (below) with 2% agar.

After 4 days of incubation at 30°C a cell suspension was made on the agar surface and used to inoculate 4 shake flasks of 2 l, each containing 400 ml of the following medium:

0.3% Yeast Extract	0.02% $MgSO_4 \cdot 7H_2O$	
0.3% Beef Extract	0.1% Urea	after sterilisation
0.3% Cornsteep Liquor	0.1% $CaCO_3$	pH 6,5
1.0% Peptone	2.0% L-Sorbose	
0.1% KH_2PO_4	ad 1 l H_2O deionized.	

After incubation at 30°C for 21 hours using 200 RPM the flasks were pooled. 1.4 l of these pooled broths were used to inoculate a jar fermenter containing 9 l medium with the following composition:

Production medium

1 % Cornsteep Liquor
0.1 % KH_2PO_4
0.01% $MgSO_4$ $7H_2O$

0.5 % $CaCO_3$
8 % L-Sorbose
1.5 % Urea
ad 1 l with deionised H_2O
After sterilisation the pH is in the range of 7.6-8.0.
The aeration during the fermentation was set to 1 vvm; the agitation to 500 RPM; the temperature to 30 °C.
After 46 hours the L-sorbose concentration of originally 70 g/l (after inoculation) had reached zero, whereas the 2-KGA concentration reached 60 g/l.
When starting with a higher L-sorbose concentration. higher yields can be obtained.

Example 2

The procedure of Example 1 is modified by using a production medium of the following composition, aeration speed of 0.5 vvm, agitation 800 r.p.m,. at pH = 7.0 (controlled with Na_2CO_3) in a 3 l (working volume 2 l) jar fermenter at 30 °C:
12.0 % L-Sorbose
1.85 % Cornsteep liquor
0.0086 % $MgSO_4.7H_2O$
0.086 % KH_2PO_4
0.086 % Urea
0.15 % Antifoam agent CA-115
After 50 hours the L-sorbose concentration of originally 110 g/l was 20 g/l, whereas the 2-KGA concentration was 81 g/l.

Claims

1. Process for producing 2-keto-L-gulonic acid by conversion of L-sorbose by means of mixed cultures of microorganisms, characterized in that a mixed culture of Gluconobacter oxydans and Bacillus megaterium is used, said mixed culture system being capable of converting L-sorbose to 2-keto-L-gulonic in a yield of at least 40 g/l.

2. Process as claimed in claim 1, characterized in that the Gluconobacter oxydans microorganism used has the following characteristics:
a) 2-Keto-L-gulonic acid is produced from sorbose,
b) ethanol is oxidized to acetic acid,
c) D-glucose is oxidized to D-gluconic acid and 2-keto-D-gluconic acid,
d) ketogenesis of polyalcohols,
e) pellicle and ring growth in mannitol broth (24 hrs cultivation) at pH 4 and 5, and pellicle growth in glucose broth at pH 4.5.

3. Process as claimed in claim 1 or 2, characterized in that the Gluconobacter oxydans microorganism used has, in addition, the following characteristics:
f) glycerol is not substantially oxidized to dihydrooxyacetone,
g) 2-keto-D-glucaric acid is produced from sorbitol and glucaric acid, but not from glucose, fructose, gluconic acid, mannitol or 2-keto-D-gluconic acid,
h) polymorphic, apparently no flagella,
i) brown pigment is produced from fructose,
j) good growth when co-cultured in the presence of Bacillus megaterium or a cell extract thereof,
k) streptomycin sensitive.

4. Process as claimed in claim 1, 2 or 3, characterized in that culture No. 2980 (DSM No. 4027), or a subculture, mutant or variant thereof is used.

5. Process according to any one of claims 1-4, characterized in that a sorbose substrate at a concentration of from about 20 to about 200 g/l, preferably from about 50 to about 100 g/l, is used.

6. Process according to claim 5, characterized in that 2-keto-L-gulonic acid is produced in a yield of at least 50 g/l, preferably at least 80 g/l.

7. Process according to any one of claims 1-6, characterized in that it is carried out at a pH between about 5 and 8, preferably between about 6 and 8.

8. Process according to any one of claims 1-7, characterized in that it is carried out at a temperature between about 25 and 35 °C, preferably at 30 ° ± 1 °C.

9. A mixed microorganism culture, having the ability to produce 2-keto-L-gulonic acid from L-sorbose in a yield of at least 40 g/l, comprising microorganisms of the species Gluconobacter oxydans and Bacillus megaterium.

10. A culture as claimed in claim 9, characterized in that the Gluconobacter oxydans microorganism has the following characteristics:
a) 2-Keto-L-gulonic acid is produced from sorbose,
b) ethanol is oxidized to acetic acid,
c) D-glucose is oxidized to D-gluconic acid and 2-keto-D-gluconic acid,
d) ketogenesis of polyalcohols,
e) pellicle and ring growth in mannitol broth (24 hrs cultivation) at pH 4 and 5, and pellicle growth in glucose broth at pH 4.5.

11. A culture as claimed in claim 9 or 10, characterized in that the Gluconobacter oxydans microorganism has, in addition, the following characteristics:
f) Glycerol is not substantially oxidized to dihydrooxyacetone,
g) 2-keto-D-glucaric acid is produced from sorbitol and glucaric acid, but not from glucose, fructose, gluconic acid, mannitol or 2-keto-D-gluconic acid,
h) polymorphic, apparently no flagella,
i) brown pigment is produced from fructose,
j) good growth when co-cultured in the presence of Bacillus megaterium or a cell extract thereof,
k) streptomycin sensitive.

12. Microorganism culture according to any one of claims 9-11, characterized by the ability to produce 2-keto-L-gulonic acid from L-sorbose in a yield of at least 50 g/l, preferably at least 80 g/l.

13. Mixed microorganism culture having the ability to produce 2-keto-L-gulonic acid from L-sorbose in a yield of at least 40 g/l comprising culture No. 2980 (DSM No. 4027), or subcultures, mutants or variants thereof.

14. A Gluconobacter oxidans strain DSM No. 4025 (CGMCC No. 0119), and sub-cultures, mutants and variants thereof, having the ability to produce 2-keto-L-gulonic acid from L-Sorbose in a yield of at least 40 g/l in a mixed microorganism culture as claimed in claim 9.

15. Bacillus megaterium strain DSM No. 4026 (CGMCC No. 0120), and sub-cultures, mutants and variants thereof, having the ability to produce 2-keto-L-gulonic acid from L-Sorbose in a yield of at least 40 g/l in a mixed microorganism culture as claimed in claim 9.

16. Process for the manufacture of ascorbic acid, characterized in that L-sorbose is converted to 2-keto-L-gulonic acid by means of a mixed culture of microorganisms as defined in claim 9 and whereby said 2-keto-L-gulonic acid obtained is then transferred to ascorbic acid in a manner known per se involving esterification, followed by enolization and lactonization.

Patentansprüche

1. Verfahren zur Herstellung von 2-Keto-L-gulonsäure durch Konversion von L-Sorbose mittels einer Mikroorganismen-mischkultur, dadurch gekennzeichnet, dass eine Mischkultur von Gluconobacter oxydans und Bacillus megaterium verwendet wird, wobei dieses Mischkulturensystem befähigt ist, L-Sorbose in 2-Keto-L-gulonsäure in einer Ausbeute von mindestens 40 g pro Liter zu überführen.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Gluconobacter oxydans die folgenden Charakteristiken aufweist:
   a) Aus Sorbose wird 2-Keto-L-gulonsäure produziert,
   b) Ethanol wird zu Essigsäur oxidiert,
   c) D-Glucose wird zu D-Gluconsäure und 2-Keto-D-gluconsäure oxidiert,
   d) Ketogenese von Polyalkoholen,
   e) Häutchen- und Ringwachstum in Mannitolbrühe (24 Stunden-kultur) bei pH 4 und 5, und Häutchenwachstum in Glucosebrühe bei pH 4,5.

3. Verfahren nach Anspruch 1 oder 2, dadurch gekennzeichnet, dass der verwendete Mikroorganismus Gluconobacter oxydans zusätzlich die folgenden Charakteristiken aufweist:
   f) Glyzerin wird nicht substantiell zu Dihydroxyaceton oxidiert,
   g) aus Sorbit und Glucarsäure wird 2-Keto-D-glucarsäure produziert, nicht aber aus Glucose, Fructose, Gluconsäure, Mannit oder 2-Keto-D-gluconsäure,
   h) Polymorphie, offensichtlich keine Flagella,
   i) aus Fructose bilden sich braune Pigmente,
   j) gutes Wachstum im Falle von Co-Kultivierung in Anwesenheit von Bacillus megaterium oder eines Zellextraktes davon,
   k) Streptomycin-sensitiv.

4. Verfahren nach einem der Ansprüche 1, 2 oder 3, dadurch gekennzeichnet, dass die Kultur Nr. 2980 (DSM No. 4027), eine Subkultur, eine Mutante oder Variante davon verwendet wird.

5. Verfahren nach einem der Ansprüche 1-4, dadurch gekennzeichnet, dass ein Sorbosesubstrat einer Konzentration von ungefähr 20 bis ungefähr 200 g pro Liter, vorzugsweise von ungefähr 50 bis ungefähr 100 g pro Liter, verwendet wird.

6. Verfahren nach Anspruch 5, dadurch gekennzeichnet, dass die 2-Keto-L-gulonsäure in einer Ausbeute von mindestens 50 g pro Liter, vorzugsweise von mindestens 80 g pro Liter produziert wird.

7. Verfahren nach einem der Ansprüche 1-6, dadurch gekennzeichnet, dass in einem pH-Bereich zwischen ungefähr 5 und 8, vorzugsweise zwischen ungefähr 6 bis 8, gearbeitet wird.

8. Verfahren nach einem der Ansprüche 1-7, dadurch gekennzeichnet, dass in einem Temperaturbereich von ungefähr 25 bis 35° C, vorzugsweise bei ca. 30° ± 1° C gearbeitet wird.

9. Eine Mikroorganismen-mischkultur mit der Fähigkeit, 2-Keto-L-gulonsäure aus L-Sorbose in einer Ausbeute von mindestens 40 g pro Liter zu produzieren, dadurch gekennzeichnet, dass die Mikroorganismen der Spezies Gluconobacter oxydans und Bacillus megaterium angehören.

10. Eine Kultur gemäss Anspruch 9, dadurch gekennzeichnet, dass der Mikroorganismus Gluconobacter oxydans die folgenden Charakteristiken aufweist:
   a) Aus Sorbose wird 2-Keto-L-gulonsäure produziert,
   b) Ethanol wird zu Essigsäure oxidiert,
   c) D-Glucose wird zu D-Gluconsäure und 2-Keto-D-gluconsäure oxidiert,
   d) Ketogenese von Polyalkoholen,
   e) Häutchen- und Ringwachstum in Mannitolbrühe (24 Stunden-kultur) bei pH 4 und 5, und Häutchenwachstum in Glucosebrühe bei pH 4,5.

11. Eine Kultur gemäss Anspruch 9 oder 10, dadurch gekennzeichnet, dass der Mikroorganismus Gluconobacter oxydans zusätzlich folgende Charakteristiken aufweist:
   f) Glyzerin wird nicht substantiell zu Dihydroxyaceton oxidiert,
   g) aus Sorbit und Glucarsäure wird 2-Keto-D-glucarsäure produziert, nicht aber aus Glucose, Fructose, Gluconsäure, Mannit oder 2-Keto-D-gluconsäure,
   h) Polymorphie, offensichtlich keine Flagella,
   i) aus Fructose bilden sich braune Pigmente,
   j) gutes Wachstum im Falle von Co-Kultivierung in Anwesenheit von Bacillus megaterium oder eines Zellextraktes davon,

k) Streptomycin-sensitiv.

12. Eine Mikroorganismenkultur gemäss einem der Ansprüche 9-11, dadurch gekennzeichnet, dass sie die Fähigkeit besitzt, 2-Keto-L-gulonsäure aus L-Sorbose in einer Ausbeute von mindestens 50 g pro Liter, vorzugsweise mindestens 80 g pro Liter zu produzieren.

13. Mischkultur mit der Fähigkeit, aus L-Sorbose 2-Keto-L-gulonsäure in einer Ausbeute von mindestens 40 g pro Liter zu produzieren, dadurch gekennzeichnet, dass es sich um die Kultur Nr. 2980 (DSM Nr. 4027), um Subkulturen, Mutanten oder Varianten davon handelt.

14. Gluconobacter oxidans-stamm DSM Nr. 4025 (CGMCC Nr. 00119) und Subkulturen, Mutanten und Varianten davon, mit der Fähigkeit aus L-Sorbose 2-Keto-L-gulonsäure in einer Ausbeute von mindestens 40 g pro Liter in einer Mischkultur gemäss Anspruch 9 zu produzieren.

15. Bacillus megaterium-stamm DSM Nr. 4026 (CGMCC Nr. 0120) und Subkulturen, Mutanten und Varianten davon, mit der Fähigkeit aus L-Sorbose 2-Keto-L-gulonsäure in einer Ausbeute von mindestens 40 g pro Liter in einer Mischkultur gemäss Anspruch 9 zu produzieren.

16. Verfahren zur Herstellung von Ascorbinsäure, dadurch gekennzeichnet, dass L-Sorbose mittels einer Mischkultur gemäss Anspruch 9 in 2-Kete-L-gulonsäure übergeführt wird, und wobei die erhaltene 2-Keto-L-gulonsäure in an sich bekannter Weise in Ascorbinsäure überführt wird, wobei von Esterifizierung, gefolgt von Enolisierung und Lactonisierung Gebrauch gemacht wird.

Revendications

1. Procédé pour la production d'acide 2-céto-L-gulonique par conversion de L-sorbose au moyen de cultures mixtes de microorganismes, caractérisé en ce qu'on utilise une culture mixte de Gluconobacter oxydans et de Bacillus megaterium, ledit système de culture mixte étant apte à convertir le L-sorbose en acide 2-céto-L-gulonique avec un rendement d'au moins 40 g/l.

2. Procédé selon la revendication 1, caractérisé en ce que le microorganisme Gluconobacter oxydans utilisé a les caractéristiques suivantes:
   a) l'acide 2-céto-L-gulonique est produit à partir de sorbose,
   b) l'éthanol est oxydé en acide acétique,
   c) le D-glucose est oxydé en acide D-gluconique et en acide 2-céto-D-gluconique,
   d) cétogénèse des polyalcools,
   e) croissance en film et en anneau dans un bouillon de mannitol (culture sur 24 heures) à pH 4 et 5, et croissance en film dans un bouillon de glucose à pH 4,5.

3. Procédé selon l'une des revendications 1 ou 2, caractérisé en ce que le microorganisme Gluconobacter oxydans utilisé a, en outre, les caractéristiques suivantes:
   f) le glycérol n'est pratiquement pas oxydé en dihydrooxyacétone,
   g) de l'acide 2-céto-D-glucarique est produit à partir de sorbitol et d'acide glucarique, mais pas à partir de glucose, fructose, acide gluconique, mannitol ou acide 2-céto-D-gluconique,
   h) polymorphique, apparemment pas de flagelles,
   i) un pigment brun est produit à partir de fructose,
   j) bonne croissance en cas de co-culture en présence de Bacillus megaterium ou d'un extrait cellulaire de celui-ci,
   k) sensible à la streptomycine.

4. Procédé selon l'une quelconque des revendications 1, 2 ou 3, caractérisé en ce qu'on utilise une culture No. 2980 (DSM No. 4027) ou une sous-culture, un mutant ou une variante de celle-ci.

5. Procédé selon l'une quelconque des revendications 1 à 4, caractérisé en ce qu'on utilise un substrat de sorbose à une concentration d'environ 20 à environ 200 g/l, de préférence d'environ 50 à environ 100 g/l.

6. Procédé selon la revendication 5, caractérisé en ce que de l'acide 2-céto-L-gulonique est produit à raison d'au moins 50 g/l, de préférence d'au moins 80 g/l.

7. Procédé selon l'une quelconque des revendications 1 à 6, caractérisé en ce qu'il est mis en oeuvr à un pH compris entre environ 5 et 8, de préférence entre environ 6 et 8.

8. Procédé selon l'une quelconque des revendications 1 à 7, caractérisé en ce qu'il est mis en oeuvre à une température comprise entre environ 25 et 35 °C, de préférence à 30 ° ± 1 °C.

9. Culture de microorganismes mixte, ayant l'aptitude à produire de l'acide 2-céto-L-gulonique à partir de sorbose à raison d'au moins 40 g/l, comprenant des microorganismes des espèces Gluconobacter oxydans et Bacillus megaterium.

10. Culture selon la revendication 9, caractérisée en ce que le microorganisme Gluconobacter oxydans a les caractéristiques suivantes:
a) l'acide 2-céto-L-gulonique est produit à partir de sorbose,
b) l'éthanol est oxydé en acide acétique,
c) le D-glucose est oxydé en acide D-gluconique et en acide 2-céto-D-gluconique,
d) cétogénèse des polyalcools,
e) croissance en film et en anneau dans un bouillon de mannitol (culture sur 24 heures) à pH 4 et 5, et croissance en film dans un bouillon de glucose à pH 4,5.

11. Culture selon l'une des revendications 9 ou 10, caractérisée en ce que le microorganisme Gluconobacter oxydans a, en outre, les caractéristiques suivantes:
f) le glycérol n'est pratiquement pas oxydé en dihydrooxyacétone,
g) de l'acide 2-céto-D-glucarique est produit à partir de sorbitol et d'acide glucarique, mais pas à partir de glucose, fructose, acide gluconique, mannitol ou acide 2-céto-D-gluconique,
h) polymorphique, apparemment pas de flagelles,
i) un pigment brun est produit à partir de fructose,
j) bonne croissance en cas de co-culture en présence de Bacillus megaterium ou d'un extrait cellulaire de celui-ci,
k) sensible à la streptomycine.

12. Culture de microorganismes selon l'une quelconque des revendications 9 à 11,caractérisée par son aptitude à produire de l'acide 2-céto-L-gulonique à partir de L-sorbose à raison d'au moins 50 g/l, de préférence d'au moins 80 g/l.

13. Culture de microorganismes mixte ayant une aptitude à produire de l'acide 2-céto-L-gulonique à partir de sorbose à raison d'au moins 40 g/l, comprenant la culture No. 2980 (DSM No. 4027), ou des sous-cultures, mutants ou variantes de celle-ci.

14. Souche de Gluconobacter oxydans DSM No. 4025 (CGMCC No. 0119) et sous-cultures, mutants et variantes de celle-ci, ayant une aptitude à produire de l'acide 2-céto-L-gulonique à partir de L-sorbose à raison d'au moins 40 g/l dans une culture de microorganismes mixte selon la revendication 9.

15. Souche de Bacillus megaterium DSM No. 4026 (CGMCC No. 0120) et sous-cultures, mutants et variantes de celle-ci, ayant une aptitude à produire de l'acide 2-céto-L-gulonique à partir de L-sorbose à raison d'au moins 40 g/l dans une culture de microorganismes mixte selon la revendication 9.

16. Procédé pour la production d'acide ascorbique, caractérisé en ce que du L-sorbose est converti en acide 2-céto-L-gulonique au moyen d'une culture mixte de microorganismes telle que définie dans la revendication 9, et que ledit acide 2-céto-L-gulonique obtenu est ensuite converti en acide ascorbique d'une manière connue en soi comprenant une estérification, suivie d'une énolisation et d'une lactonisation.